Detection of Airborne Asbestos by Fluorescent-labeled protein probe and its Application to quick Monitoring

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Asbestos: silicate mineral fiber

serpentine

chrysotile
$\text{Mg}_6\text{Si}_4\text{O}_{10}(\text{OH})_8$

crocidolite
$\text{Na}_2(\text{Fe}^{3+})_2(\text{Fe}^{2+})_3\text{Si}_8\text{O}_{22}(\text{OH})_2$

amphibole

amosite
$(\text{Fe, Mg})_7\text{Si}_8\text{O}_{22}(\text{OH})_2$
Asbestos: widely used in construction materials

- Fire retardant
- Slate roof
- Dry wall (Gypsum)
- Heat insulator

東日本大震災NEVER特設ページより
Asbestos: lung cancer and mesothelioma


Asbestos exposure


30 - 40 years latent period

mesothelioma

Japan shock 2005

This has come largely as a shock to the Japanese public in 2005. Not all those deaths were workers; many of the deaths were people who lived near factories including family members of workers.
Amount of asbestos in USA and Japan

5 million tons of asbestos are remained in Japan.
Worldwide trends in the mesothelioma

<table>
<thead>
<tr>
<th>Country or Region</th>
<th>Incidence cases/million population</th>
<th>Predicted Peak Year</th>
<th>Predicted No. of Deaths in Next 40 Yr</th>
<th>Predicted Cost billions of U.S. dollars</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>15</td>
<td>2004</td>
<td>72,000</td>
<td>200</td>
</tr>
<tr>
<td>Europe</td>
<td>18§</td>
<td>2015–2020</td>
<td>250,000</td>
<td>80</td>
</tr>
<tr>
<td>Japan</td>
<td>7</td>
<td>2025</td>
<td>103,000</td>
<td>—</td>
</tr>
<tr>
<td>Australia</td>
<td>40</td>
<td>2015</td>
<td>30,000</td>
<td>5–10</td>
</tr>
</tbody>
</table>

* The sources of the data on the incidence (most recent figures), predicted peak year, and predicted number of deaths in the next 40 years are as follows: United States, Roushdy-Hammady et al.²; Europe, Pelin et al.³; Japan, Sebastien et al.⁴; and Australia, Wagner et al.⁵ The sources of the data on predicted cost are as follows: United States, Shah and Williams⁶; Europe, Lee et al.⁷; and Australia, Wagner et al.⁵ Costs for Japan are unknown.
† The predicted number of deaths is estimated from data on annual incidence and predicted peak year.
‡ The costs shown are for compensation only; health care costs are excluded.
§ The incidence, in number of cases per million population, is 33 in Great Britain, 30 in the Netherlands, 15 in Germany, 16 in France, and 19 in Italy (range in Europe, 15 to 33).

Why rapid detection method of asbestos is required?
Conventional method for airborne asbestos

Membrane filter

Treat with acetone vapor (transparency)

Asbestos

Pump

Phase contrast microscopy (PCM)
Phase contrast microscopy (PCM)
Asbestos monitoring (Ministry of Environment)

【Phase contrast microscopy】

Total fiber concentration under PCM

Less than 1 fiber/L  OK

More than 1 fiber/L  Asbestos or not?

【Electron microscopy wit EDX】

Time consuming and expensive
Asbestos risk at demolition site

- More than 100 million tons of materials containing asbestos will be dumped until 2035.
- Demolition will be completed within a couple of days.
- Rapid detection method of asbestos is required.
Airborne asbestos from earthquake debris?
Quick detection of asbestos

Fluorescent
Asbestos-binding protein

asbestos
Asbestos-binding proteins
Asbestos-binding protein

Protein sources

Mouse lung  Escherichia coli

Asbestos-binding protein!
DksA: chrysotile-binding protein

Kd = 3.5 nM

Enzyme asbestos detection

Alkaline phosphatase (AP)

Asbester™

Asbestos (Chrysotile)

Asbestos (Chrysotile)

<table>
<thead>
<tr>
<th>0</th>
<th>0.005</th>
<th>0.01</th>
<th>0.05</th>
<th>0.1</th>
<th>0.5</th>
<th>1   (mg)</th>
</tr>
</thead>
</table>

![Image of DksA molecule with Kd value]

![Image of Enzyme asbestos detection process]

![Image of Asbestos detection results]
Chrysotile detection in the materials

1. Construction materials + Asbestos™

2. Centrifuge and remove supernatant

3. Extraction of Asbestos™

4. Transfer supernatant to a new tube

5. Addition of substrate

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**Example**

**Dry wall** (asbestos or non-asbestos)

<table>
<thead>
<tr>
<th>Non</th>
<th>3.5%</th>
<th>Non</th>
<th>3.0%</th>
</tr>
</thead>
</table>

---

**Chrysotile content (%)**

- A 600
- 0
- 0.2
- 0.4
- 0.6
- 0.8
- 1

**Chrysotile content (%)**

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Specificity of Asbester (DksA-AP fusion)

<table>
<thead>
<tr>
<th>Materials</th>
<th>Composition</th>
<th>Bind</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysotile</td>
<td>$\text{Mg}_6\text{Si}<em>4\text{O}</em>{10}(\text{OH})_8$</td>
<td>+</td>
</tr>
<tr>
<td>Antigorite</td>
<td>$\text{Mg}_6\text{Si}<em>4\text{O}</em>{10}(\text{OH})_8$</td>
<td>-</td>
</tr>
<tr>
<td>Talc</td>
<td>$\text{Mg}_3\text{Si}<em>4\text{O}</em>{10}(\text{OH})_2$</td>
<td>-</td>
</tr>
<tr>
<td>Amosite</td>
<td>$(\text{Mg,Fe})_7\text{Si}<em>8\text{O}</em>{22}(\text{OH})_2$</td>
<td>-</td>
</tr>
<tr>
<td>Crosidolite</td>
<td>$\text{NaFe}^{+3}\text{Fe}^{+2}_3\text{Si}<em>8\text{O}</em>{22}(\text{OH})_2$</td>
<td>-</td>
</tr>
<tr>
<td>Glass wool</td>
<td>$\text{CaO-P}_2\text{O}_5-\text{SiO}_2-\text{Al}_2\text{O}_3$</td>
<td>-</td>
</tr>
<tr>
<td>Silica</td>
<td>$\text{SiO}_2$</td>
<td>-</td>
</tr>
<tr>
<td>Titanium oxide</td>
<td>$\text{TiO}_2$</td>
<td>-</td>
</tr>
<tr>
<td>Silicon carbide</td>
<td>$\text{SiC}$</td>
<td>-</td>
</tr>
<tr>
<td>Magnesium hydro</td>
<td>$\text{Mg(OH)}_2$</td>
<td>-(/-/+)</td>
</tr>
<tr>
<td>Cement</td>
<td>$\text{SiO}_2, \text{CaO, etc}$</td>
<td>-</td>
</tr>
<tr>
<td>Rockwool</td>
<td>$\text{SiO}_2, \text{CaO, etc}$</td>
<td>-</td>
</tr>
<tr>
<td>Dry wall</td>
<td>$\text{CaSO}_4$</td>
<td>-</td>
</tr>
</tbody>
</table>
(b) after 250 ps
(d) after 3 ns
How does DksA recognize asbestos?

Mg₆Si₄O₁₀(OH)₈

Chrysotile

Mg₆Si₄O₁₀(OH)₈

Antigorite

5.5 nm

2-3 nm

Mg-OH

SiO₂
HNS: amphibole asbestos-binding protein

- Amphibole asbestos
- Wollastonite
- Aluminum silicate
- Titanium oxide
- Silicon carbide

Amphibole asbestos (Amosite, Crocidolite, etc)

Asbestos (amphibole asbestos)-binding region
## Specificity

<table>
<thead>
<tr>
<th>Fiber</th>
<th>DksA</th>
<th>HNS (modified)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asbestos</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysotile</td>
<td>Bound</td>
<td></td>
</tr>
<tr>
<td>Crocidolite</td>
<td>Bound</td>
<td></td>
</tr>
<tr>
<td>Amosite</td>
<td>Bound</td>
<td></td>
</tr>
<tr>
<td>Anthophyllite</td>
<td>Bound</td>
<td></td>
</tr>
<tr>
<td>Tremolite</td>
<td>Bound</td>
<td></td>
</tr>
<tr>
<td>Actinolite</td>
<td>Bound</td>
<td></td>
</tr>
<tr>
<td>Glass wool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine glass fiber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rockwool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fire proof fiber (RF1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fire proof fiber (RF2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminum silicate fiber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titanium potassium</td>
<td>Slightly bound</td>
<td></td>
</tr>
<tr>
<td>Silicon carbide whisker</td>
<td>Bound</td>
<td>Bound</td>
</tr>
<tr>
<td>Titanium oxide whisker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wollastonite</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Detection of asbestos under fluorescence microscopy
Fluorescent microscopy (FM)
Modification of protein with fluorescence

Fluorescence molecule

Asbestos-binding protein

Fluorescein

491nm

521nm

Asbestos
Detection of airborne asbestos under FM

Filter membrane

asbestos

One drop of fluorescent-label protein

Conventional

PCM

New

FM
30 nm single chrysotile fibril was detected under FM.
Double staining of asbestos

DksA-Cy3 (red)

HNS-FITC (green)
Combination of phase-contrast and fluorescence microscopy (PCM-FM fusion)
Fluorescent microscopy

Light for FM

Light for PCM
Detection of Airborne Asbestos by Fluorescent-labeled protein probe and its Application to quick Monitoring

1. We discovered asbestos-binding proteins.
2. We developed a fluorescence microscopy-based method for selective and highly sensitive detection of two different types of asbestos.
3. The diameter of the thinnest asbestos fibers visualized under fluorescence microscopy was 30-35 nm.
4. Then we proposed PCM and FM fusion analysis.
5. This method could be used for on-site quick monitoring of airborne asbestos, for example, during demolition work.